The Effect of Ora Salts™ on the Growth of *Lactobacillus acidophilus* and *Streptococcus mutans* Bacteria *in-vitro*

Yumna Ebrahim

(Student number: 200901957)

Postal address: University of Johannesburg, PO BOX 524, Auckland Park, 2006 South Africa

ABSTRACT

Micro-colonies of bacteria occur on the oral mucosa, the tongue and tooth surfaces below and above the gingival margin (Dewhirst *et al.*, 2010). Regular oral care aids in reducing microbial accumulation, preventing conditions such as caries, gingivitis and periodontal disease that arise due to microbial build-up (Gurenlian, 2007). The percentage prevalence of dental caries is 95 % in South African adults (35-44 years) and 68% of South African adults have not had their caries treated (van Wyk, 2004). Poor management of gingivitis increases potential progression to peridontitis (Arndt, 2010). Microbial analyses of periodontal disease and oral colonization reveal *Streptococcus mutans* to be amongst dominant pathogenic bacteria (Contardo *et al*,. 2011). Peridontitis is treated with non-surgical mechanical debridement and oral maintenance care (Heitz-Mayfield, 2009). Metronidazole and/or amoxicillin antibiotics are frequently prescribed for peridontitis (Heitz-Mayfield, 2009). Side effects of these include nausea, vomiting and diarrhoea, while long term usage may pose a threat to liver health and lead to teeth removal (NHS, 2012). Antibiotics cause destruction to both commensal (e.g. *Lactobacillus acidophilus)* and pathogenic bacteria (e.g. *Streptococcus mutans),* which may render individual susceptible to infections and diseases through disruption of perpetual homeostasis (Blaser, 2011).

Ora Salts™ is a mouth wash with homoeopathic remedies that contains no sugar, alcohol, artificial ingredients, preservatives or colourants (Oralink, 1997). It remains to be proven whether it has significant *in-vitro* activity against the pathogenic microorganism *Streptococcus mutans,* together with a preserving action on the commensal micro-organism *Lactobacillus acidophilus*.

The primary objective of the study was to expose pathogenic microorganism *Streptococcus mutan*s and commensal microorganism *Lactobacillus acidophilus* to Ora Salts™, saline solution and distilled water to determine their efficacy *in-vitro* on the above mentioned microorganisms.

This qualitative *in-vitro* control study was conducted at the Water and Health Research unit at the University of Johannesburg, Doornfontein campus, under the supervision of qualified laboratory technicians with relevant permission granted. *Lactobacillus acidophilus* and

Streptococcus mutans were tested against distilled water, saline solution and Ora Salts™. These bacterial organisms (*L. acidophilus* and *S. Mutans)* were grown and then adjusted to the turbidity of 0.5 McFarland standard to get equal growth amounts as per explanation in Appendix B. Agar plates were then streaked using the sterile swab with *L. acidophilus* and *S. mutans*, and the plates were allowed to dry for approximately 5 minutes. The freshly prepared Ora Salts™ solution, distilled water and saline-impregnated disks were placed on the surface of the agar, using flame-sterilized forceps to dispense each antimicrobial disk one at a time until all disks were placed. The plates were then inverted and placed in a 35°C incubator for 48hours. Each plate was numbered before incubation to allow identification of the contents progress, and once the plates were incubated, they were checked for a zone of inhibition around each of the impregnated discs. Two more experiments were conducted based on an adaption to this methodology. For the first adaptation experiment, a two times and five times Ora Salts™ increased strength was tested against *S. mutans* and *L. acidophilus*. Adaptation experiment two involved exposing *S. mutans* and *L. acidophilus* to one time, two times and five times strength Ora Salts™ for five minutes instead of two minutes as described by the manufacture. These experiments followed the same procedure mentioned above.

It was hypothesized that Ora Salts™ would inhibit the growth production *in-vitro* of *S. mutans* and *L. acidophilus*. This could be illustrated by exposing clear zones of inhibition in experimental plates. The disc diffusion method and two adaptation disc diffusion experiments were done to test the stated hypothesis of this study.

There were no recorded zones of inhibition in any of the utilised solutions for all the experiments. The results indicate that the concentration of Ora Salts™ tested does not directly inhibit growth of *S. mutans* and *L. acidophilus* grown to 0.5 McFarland standard; however, results are not conclusive, as further investigation into the homoeopathically prepared remedies on the physiology of living tissue are required.

Introduction

Definition

Caries, gingivitis and periodontal disease arise due to microbial build-up (Gurenlian, 2007). Microbial analyses of periodontal disease and oral colonization reveal *Streptococcus mutans* to be amongst dominant pathogenic bacteria (Contardo *et al*,. 2011).

Epidemiology

The percentage prevalence of dental caries include 95% adults aged between 35-44 years old, and 68% are not treated for caries in South Africa (van Wyk, 2004), while the poor management of gingivitis increases potential progression to peridontitis (Arndt, 2010).

Aetiology

Of the 700 various bacterial species contained in the human oral cavity, majority of these microorganisms are associated with dental plaque (He and Shi., 2009). Accumulated plaque on dental surfaces, composed of oral flora is the primary causative factor of dental caries (Chandrabhan, 2012). Regular oral care aids in reducing microbial accumulation, preventing conditions such as caries, gingivitis and periodontal disease that arise due to microbial build-up (Gurenlian, 2007).

Signs and symptoms

As cavities extend deeper into the teeth, increased sensitivity to food may occur, although smaller cavities may be asymptomatic (Horne, 2014). Light or dark brown spots on anterior teeth allow cavities to be diagnosed through visual diagnosis (Horne, 2014). Light brown cavities depicts 'fast-growing' cavity, and dark brown 'slower-growing' cavity (Horne, 2014). As the cavity growth advances, part of the afflicted tooth may break and leave a hole (Horne, 2014).

Symptoms of gingival disease include :

- Persistent foetid breath
- Gingival area inflamed, with rubor
- Tender gingiva that may bleed
- Mastication is painful
- Teeth that are loose and sensitive
- Receding gums or teeth that appear longer

(NIDCR, 2013).

Treatment

Peridontitis is treated with non-surgical mechanical debridement and oral maintenance care (Heitz-Mayfield, 2009). Metronidazole and/or amoxicillin antibiotics are frequently prescribed for peridontitis (Heitz-Mayfield, 2009). Side effects of these include nausea, vomiting and diarrhoea, while long term usage may pose a threat to liver health and lead to teeth removal (NHS, 2012). Antibiotics cause destruction to both commensal (e.g. *Lactobacillus acidophilus)* and pathogenic bacteria (e.g. *Streptococcus mutans),* which may render individual susceptible to infections and diseases through disruption of perpetual homeostasis (Blaser, 2011).

Ora Salts™ is a mouth wash with homoeopathic remedies that contains no sugar, alcohol, artificial ingredients, preservatives or colourants (Oralink, 1997). It remains to be proven whether it has significant *in-vitro* activity against the pathogenic microorganism *Streptococcus mutans,* together with a preserving action on the commensal micro-organism *Lactobacillus acidophilus*.

Oral hygiene procedures, including anti microbial mouth rinses, help to control biofilm growth on the oral mucosa (Gurenlian, 2007).

Method and design

Research design

This was a qualitative *in-vitro* controlled study, that was conducted at the Water and Health Research unit at the University of Johannesburg, Doornfontein campus, under the supervision of qualified laboratory technicians with relevant permission granted. *Lactobacillus acidophilus* and *Streptococcus mutans* were tested against distilled water, saline solution and Ora Salts™ in controlled labratory conditions.

Bacterial Organisms, and their selection

Streptococcus mutans (S. mutans) is the principal etiological organism that causes dental caries (Chandrabhan *et al*., 2012), and is actively involved in the disease (He and Shi, 2009). When the glucose polymer glucan is produced by *S. mutans*, a biofim which confines oral bacteria, food debris and salivary components, is formed (Ito *et al*., 2011). *S. mutans* produces acids in the biofilm that demineralize the tooth surface, progressing to dental caries (Ito, *et al.,* 2011). Commensal bacteria, *Lactobacillus acidophilus (L. acidophilus),* aids to decrease to dental caries formation in reducing adhesion of pathogenic bacteria to teeth surfaces (Tahmourespour and Kermanshahi, 2011).

Method

Lactobacillus acidophilus and *Streptococcus mutans* were tested against distilled water, saline solution and Ora Salts™. These bacterial organisms (*L. acidophilus* and *S. Mutans)* were grown and then adjusted to the turbidity of 0.5 McFarland standard to get equal growth amounts as per explanation in Appendix B. Agar plates were then streaked using the sterile swab with *L. acidophilus* and *S. mutans,* and the plates were allowed to dry for approximately 5 minutes. The freshly prepared Ora Salts™ solution, distilled water and saline-impregnated disks were placed on the surface of the agar, using flame-sterilized forceps to dispense each antimicrobial disk one at a time until all disks were placed. The plates were then inverted and placed in a 35°C

incubator for 48hours. Each plate was numbered before incubation to allow identification of the contents progress, and once the plates were incubated, they were checked for a zone of inhibition around each of the impregnated discs. Two more experiments were conducted based on an adaption to this methodology. For the first adaptation experiment, a two times and five times Ora Salts™ increased strength was tested against *S. mutans* and *L. acidophilus*. Adaptation experiment two involved exposing *S. mutans* and *L. acidophilus* to one time, two times and five times strength Ora Salts™ for five minutes instead of two minutes as described by the manufacture. These experiments followed the same procedure mentioned above.

Data analysis

During the preparation for this research study, it was determined that the ANOVA Statistical method was to be used to analyse the data. However, statistical analysis was not required as experiments did not illustrate any zones of inhibition. Thus, the ANOVA method could not be implemented , due to experimental values being zero (0.00mm).

Results and Discussion

In a comparative analysis, experimental plates, saline plates as well as distilled water plates exhibited no zones of inhibition. A possible explanation, as mentioned above, could be the absence of the cumulative characteristics oral cavity contains. The human oral cavity encompasses certain environmental conditions together with interactions between microorganisms of the oral cavity as well as a particular bacterial flora (Krzyściak *et al*., 2014). Through these natural conditions are oral biota best investigated (Krzyściak *et al*., 2014). With *in-vitro* testing, many biological factors are omitted and thus a true reflection may not be presented.

Another possibility of research resulting in no antibacterial efficacy, could be that the bacteria tested in this study may be salt-resistant, therefore the bacteria not responding to the Ora Salts™. Research was done to investigate the hyperosmotic stress response of *S. mutans*. Results

included 'cross-talk between osmotic and oxidative stress responses in *S. mutans*' (Abranches *et al*., 2006). The article by Abranches *et al*., (2006), elaborates that an osmotic upshift obliges bacteria to alter their physiology through triggering or deactivating specific enzymes and transporters, together with modifying patterns of gene expression to preserve water balance (Abranches *et al*., 2006). *S. mutans* adapts to rapidly increasing responses and environmental stimuli and has developed multiple pathways toward stressors (Abranches *et al*., 2006).

All the plates for the solutions (Ora Salts™, saline solution and distilled water) tested had good growth for *S. mutans* and *L. acidophilus* (see Figure 4.3.1.1and 4.3.1.2) . This indicates favourable agar, due to good bacterial growth on the plates. Poor growth of the organisms could affect the efficacy of the study.

Conclusion

There were no recorded zones of inhibition in any of the utilised solutions for all the experiments. The results indicate that the concentration of Ora Salts™ tested does not directly inhibit growth of *S. mutans* and *L. acidophilus* grown to 0.5 McFarland standard; however, results are not conclusive, as further investigation into the homoeopathically prepared remedies on the physiology of living tissue are required.

Acknowledgements

This study was conducted by the author under the supervision of the supervisor Dr Tsele and co-supervisor Vusi Ntema in partial fulfilment for the Master's Degree in Technology: Homoeopathy. The support, encouragement and advice that the supervisor and co-supervisor aided the author with, did not go unnoticed.

References

Abranches, J., Lemos, J and Burne, R. (2006). Osmotic stress responses of Streptococcus mutans. *FEMS Microbiology Letters*. 255 (2), 240-246.

Arndt, H. (2010). It's time to revisit gingivitis. *Proquest*. 1 (1), 56-59.

Blaser, M. (2011). Antibiotic overuse: Stop the killing of beneficial bacteria. *Nature*. 476 (1), 393–394 .

Chandrabhan, D., Hemlata, R., Renu, B and Pradeep, V. (2012). Isolation of Dental Caries Bacteria from Dental Plaque and Effect of Tooth Pastes on Acidogenic Bacteria. *Open Journal of Medical Microbiology*. 2 , 65-69.

Contardo, M., Díaz, N., Lobos, O., Padilla, C and Giacaman, R. (2011). Oral colonization by *Streptococcus mutans* and its association with the severity of periodontal disease in adults. *Science Direct*. 4 (1), 9-12.

Dewhirst, F., Chen, T., Izard, J., Paster, B., Tanner, A., Yu, W., Lakshmanan, A and Wade, G. (2010). The Human Oral Microbiome. *Journal of Bacteriology*. 19 (192), 5002-5017.

Gurenlian, J. (2007). The role of dental plaque biofilm in oral health. *Journal of Dental Hygiene*. 1 (1), 4.

He, X and Shi, W. (2009). Oral Microbiology: Past, Present and Future. *International Journal of Oral Science*. 1 (2), 47-58.

Heitz-Mayfield, L. (2009). Systemic antibiotics in periodontal therapy. *Australian Dental Journal*. 54 (1), 96-101.

Horne, S. (2014). *Cavities.* Available: http://www.medicinenet.com/cavities/page3.htm. (Accessed 10 March 2015).

Ito, K., Ito, S., Shimamura, T., Weyand, S., Kawarasaki, Y., Misaka, T., Abe, K., Kobayashi, T., Cameron, A and Iwata, S. (2011). Crystal Structure of Glucansucrase from the Dental Caries Pathogen *Streptococcus mutans*. *Journal of Molecular Biology*. 408 (2), 177-186.

Krzyściak, W., Jurczak, A., Kościelniak, D., Bystrowska, B & Skalniak, A. (2014). The virulence of Streptococcus mutans and the ability to form biofilms. *European Journal of Clinical Microbiology*. 55 (33), 499-515.

NHS, National Health Service. (2012). *Gum disease - treatment.* Available: http://www.nhs.uk/Conditions/Gum-disease/Pages/Treatment.aspx. (Accessed 15 September 2013).

NIDCR, National Institute of Dental and Craniofacial Research. (2013). *Periodontal (Gum) Disease: Causes, Symptoms, and Treatments.* Available: http://www.nidcr.nih.gov/oralhealth/Topics/GumDiseases/PeriodontalGumDisease.htm. (Accessed 21 April 2015).

OraLink (1997). *Ora Salts.* Available: http://www.oralinkhealth.com/ora-range.html. (Accessed 10 February 2014).

Tahmourespour, A and Kermanshahi, R. (2011). The effect of a probiotic strain (*Lactobacillus acidophilus*). *Bosnian Journal of Basic Medical Sciences*. 11 (1), 37-40.

van Wyk, P. (2004). Oral health in South Africa. *International Dental Journal*. 54 (1), 373-377.